

US Serial No. 07/565,673
Page 2

meaning of *Festo*¹. The Remarks begin on page 5, while the Conclusion is on page 8.

LIST OF CLAIMS, SHOWING THE STATUS OF EACH CLAIM

In the following Claims, underlining denotes added text, while strikethrough denotes deleted text.

Claims 1-47. (Cancelled)

48. (Currently Amended) A method of obtaining a non-reverting mutant alkalophilic Bacillus strain wherein said alkalophilic Bacillus strain is Bacillus novo species PB92 or the derivative PBT 110, having a reduced level of a wild-type high alkaline serine protease, said method comprising the steps of:

a) transforming an alkalophilic Bacillus strain comprising a gene encoding the wild-type alkaline serine protease with a cloning vector comprising DNA encoding a replication function and 5' and 3' flanking non-coding regions of said gene encoding the wild-type high alkaline serine protease but not the coding region of said gene encoding the wild-type high alkaline serine protease gene, wherein a sufficient amount of said 5' and 3' flanking non-coding regions is present to provide for homologous recombination with the indigenous gene encoding the wild-type alkaline serine protease of said alkalophilic Bacillus strain whereby transformants having an inactivated wild-type Bacillus PB92 extracellular serine protease ~~reduced level of said wild-type alkaline serine protease~~ are obtained;

b) growing said transformants under conditions whereby the replication function encoded by said cloning vector is inactivated; and
and

c) isolating transformants having a reduced level of the wild-type alkaline serine protease, wherein the level of wild-type alkaline serine protease is not detectable.

¹ *Festo Corp. v. Shoketsu Kogyo Kabushiki Co.*, No. 95-1066, 2000 WL 1753646 (Fed. Cir. Nov. 29, 2000).

US Serial No. 07/565,673

Page 3

49. (Cancelled)

50. (Once Amended) A mutant, non-reverting alkalophilic Bacillus strain wherein said mutant alkalophilic Bacillus strain is Bacillus novo species PB92 or the derivative PBT 110, producing a mutant high alkaline serine protease and no detectable level of a wild-type high alkaline serine protease, wherein said mutant, non-reverting alkalophilic Bacillus strain is obtained by growing an alkalophilic Bacillus strain which comprises an inactivated wild-type serine protease gene, such that said Bacillus strain is incapable of producing said wild-type high alkaline serine protease, and wherein said Bacillus strain is transformed with a plasmid expression vector comprising said mutant high alkaline serine protease gene, and further wherein said gene encoding the mutant high alkaline serine protease comprises a replacement of at least one amino acid residue in the nucleotide sequence encoding the wild type high alkaline serine protease of Bacillus novo species PB92 or derivative thereof, and wherein said replacement is at an amino acid residue position selected from the group consisting of positions 160, 216, and 212.

51. (Cancelled)

52. (Cancelled)

53. (Previously Presented) The alkalophilic Bacillus strain of Claim 50 wherein said strain is asporogenic.

54. (Currently Amended) A method for the production of a mutant high alkaline protease, said method comprising the steps of:

a) obtaining an alkalophilic Bacillus host selected from the group consisting of Bacillus novo species PB92 and its derivatives wherein said derivatives retain the characteristics of Bacillus novo species PB92 and said alkalophilic Bacillus host is incapable of producing a wild-type high alkaline serine protease, and comprises a chromosomal deletion of the gene encoding an the wild-type high alkaline serine protease;

GC329 Final Office Action Response 12-1-04

US Serial No. 07/565,673
Page 4

b) transforming said alkalophilic *Bacillus* host with an integration cassette comprising a gene encoding a mutant high alkaline serine protease, wherein said gene encoding the mutant high alkaline serine protease comprises a replacement of at least one amino acid residue in the nucleotide sequence encoding the wild type high alkaline serine protease of *Bacillus novo* species PB92 or derivative thereof to obtain a non-reverting mutant alkalophilic strain, such that said alkalophilic *Bacillus* host produces no detectable level of wild-type serine protease activity, and wherein said replacement is at an amino acid residue position selected from the group consisting of positions 160, 216, and 212; and

c) growing said mutant alkalophilic *Bacillus* host under conditions whereby said mutant high alkaline serine protease is expressed.

55. (Cancelled)

GC329 Final Office Action Response 12-1-04

BEST AVAILABLE COPY